nucleotide-gated (CNG) ion channels. CNG channels are cation channels modulated by cytoplasmic cyclic nucleotides, which play important roles in sensory signal transduction in eukaryotes. Cell-based assays have accumulated evidence for lipid-modulation of CNG ion channels. To isolate and systematically study the effect of lipids on CNG channels, we reconstituted a model CNG channel into lipid bilayer nanodiscs composed of phosphocholine, phosphoglycerol and phosphoethanolamine lipids. We characterized these nanodisc preparations using size exclusion chromatography, electron microscopy and dynamic light scattering to ensure the formation of nanosized discoidal protein/lipid complexes. The reconstitution of CNG channels into nanodiscs permits the direct thermodynamic measurement of cAMP binding to the channel using isothermal titration calorimetry (ITC). This platform allowed us to probe the binding of cyclic nucleotides to CNG channels in the presence of different lipids, an important step in understanding the molecular mechanism of lipid modulation of CNG channel gating.

Cardiac Muscle Mechanics and Structure I

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Selective Alpha7 Nicotinic Receptor Agonist Increases Cardiac Function in Isolated Mouse Hearts by a Non-Nicotinic Mechanism

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Selective alpha7 nicotinic receptor agonists, such as GTS-21, are known to have neuroprotective and anti-inflammatory actions, but the effect of these agents on the heart is not known. Therefore, we evaluated the actions of GTS-21 in isolated atria and isolated hearts from C57BL/6 mice. GTS-21 (1-100 μM) caused a concentration-dependent increase in amplitude of contractions and decrease in beating rate in isolated atria. Percent changes in rate and contractile force evoked by 30 μM GTS-21 were −14 ± 2 and +36 ± 11, respectively (n = 4). The onset of responses to GTS-21 was slower than would occur to acetylcholine and norepinephrine, and effects of GTS-21 were not blocked by atropine or atenolol. The impact of GTS-21 on left ventricular function was evaluated next in isolated hearts paced at 400 BPM. Treatment with 30 μM GTS-21 caused significant increases in contractile function: developed pressure (+30 ± 4%); peak systolic pressure (+16 ± 2%); dP/dtmax (+31 ± 6%). Diastolic pressure was also decreased (−60 ± 10%) while the rate of relaxation increased (-dP/dtmax, +36 ± 6%) with no sizable change in coronary perfusion pressure ($p < 0.05$ for all parameters, n = 4). Surprisingly, responses to GTS-21 were not affected by a selective alpha7 antagonist or the nonselective nicotinic antagonist mecamylamine, and there was no evidence of desensitization. Our data demonstrate that GTS enhances atrial and ventricular contractility and improves LV relaxation with a modest decrease in heart rate. GTS-21 cardiac actions occur by a mechanism that is independent of nicotinic receptors and autonomic nerves. The unique profile of cardiac effects for GTS-21 suggests its potential use in the setting of acute heart failure.

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Life-Long Treatment with Late Sodium Current Blocker Reduces Myocardial Dysfunction and Remodeling in a Mouse Model of Hypertrophic Cardiomyopathy

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No drugs are capable to prevent phenotype development and adverse cardiac remodeling in hypertrophic cardiomyopathy (HCM). Ranolazine, a late Na^+ current blocker, reduced arrhythmogenicity and improved relaxation in cardiomyocytes and trabeculae from HCM patients (Coppini et al., Circulation 2013). Employing a transgenic mouse model carrying the HCM-associated R92Q mutation in the *TNNT2* gene, we previously showed that acute in vitro treatment with ranolazine is capable to reverse some electromechanical alterations, including the prolonged kinetics of Ca^{2+} transients, the higher diastolic [Ca^{2+}] and the increased frequency of arrhythmogenic spontaneous activity (Pioner et al., Biophys J 2014, 106, 644a). Here we employed the same mouse model to assess whether long-term oral treatment with ranolazine since birth is capable to prevent the HCM phenotype and the associated myocardial remodeling. We compared the behavior of WT, R92Q-untreated and R92Q-treated 1 year old mice. Echocardiographic measurements showed that the R92Q-treated in vivo hearts lacked the left ventricular hypertrophy, hypercontractility and diastolic dysfunction found in the R92Q-untreated mice. Gadolinium-contrast magnetic resonance showed that the intramyocardial fibrosis of the R92Q-untreated hearts was largely reduced in the treated mice.Both amelioration of cardiomyocyte function and reduction of extracellular fibrosis may contribute to the positive effect of the long-term treatment with ranolazine that could represent a candidate for preventive treatment of phenotype-negative mutation carriers. Mechanical experiments in intact left and right ventricular trabeculae confirmed the alterations we had previously reported in R92Q-untreated mice compared to WT and showed that those alterations were mostly reversed in the R92Q-treated mice. In the R92Q-treated preparations the inotropic response to isoproterenol was preserved and the occurrence of spontaneous activity was markedly reduced compared to the untreated trabeculae and was comparable to that of WT trabeculae.

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Stage-Dependent Benefits and Risks of Pimobendan in Genetic Dilated Cardiomyopathy Mice with Progressive Heart Failure

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The Ca^{2+} sensitizer pimobendan is a unique inotropic agent that, compared with traditional inotropes, improves cardiac contractility with less oxygen consumption and potentially fewer adverse effects on myocardial remodelling and arrhythmia. However, clinical trials report contradictory effects of pimobendan in heart failure (HF) patients. We provide mechanistic experimental evidence of the efficacy of pimobendan using a novel mouse model of progressive HF.A knock-in mouse model of human genetic dilated cardiomyopathy, which shows a clear transition from compensatory to end-stage HF at a fixed time during growth, was used to evaluate the efficacy of pimobendan and explore the underlying molecular and cellular mechanisms.Pimobendan prevented myocardial remodelling in compensated HF and significantly extended the life span in both compensated and end-stage HF, but dose-dependently increased the sudden death in end-stage HF. In cardiomyocytes isolated from end-stage HF mice, pimobendan induced triggered activity probably due to early or delayed afterdepolarizations via markedly up-regulated electrogenic sodium/calcium exchanger 1 activation. The L-type Ca^{2+} channel blocker verapamil decreased the incidence of triggered activity, suggesting that this was from overly elevated cytoplasmic Ca^{2+} through increased Ca^{2+} entry by phosphodiesterase 3 inhibition under diminished sarcoplasmic reticulum Ca^{2+} reuptake and increased Ca^{2+} leakage from sarcoplasmic reticulum in end-stage HF.Pimobendan is beneficial irrespective of HF stage, but increases sudden cardiac death in end-stage HF with extensive remodelling ofCa^{2+} handling. Reduction of cytoplasmic Ca^{2+} elevated by phosphodiesterase 3 inhibition may decrease the risk of sudden cardiac death.

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Saxagliptin Preserves Cardiomyocyte Function and Morphology in Aortic-Banded Mini-Swine

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Impaired cGMP-PKG signaling may contribute to cardiomyocyte remodeling and cardiac dysfunction in heart failure with preserved ejection fraction (HFpEF) patients. The purpose of this study was to assess cardiomyocyte function and morphology in aortic-banded mini-swine displaying a HFpEF phenotype following manipulation of cGMP signaling via two mechanisms: 1) driving cGMP synthesis with the DPP4 inhibitor saxagliptin; and 2) preventing cGMP catabolism via the PDE5 inhibitor tadalafil. We hypothesized that preserving cGMP-PKG signaling would prevent cardiomyocyte dysfunction and remodeling. Contractile function was measured in enzymatically isolated cardiomyocytes electrically stimulated at three frequencies (0.25, 0.5, 1.0 Hz)from four groups: control (CON), aortic-banded (AB), AB saxagliptin-treated (AB-SAX), and AB tadalafil-treated (AB-TAD). Increased cGMP activity was only observed in AB-TAD animals, however, neither drug treatment increased PKG activity. A significant pacing-induced decrease in diastolic sarcomere length was observed in the AB and AB-TAD groups compared to CON. This finding, indicative of impaired diastolic relaxation, was prevented by saxagliptin treatment. Shortening amplitude